TRAVAUX DU MUSÉUM NATIONAL D'HISTOIRE NATURELLE «GRIGORE ANTIPA»

THE JOURNAL OF "GRIGORE ANTIPA" NATIONAL MUSEUM OF NATURAL HISTORY

Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa" 66 (1): 113–133 (2023)
doi: 10.3897/travaux.66.e96322

RESEARCH ARTICLE

The first sighting of *Isophya posthumoidalis* (Orthoptera, Tettigoniidae) in Ukraine and observations on its biology and acoustics

Andriy Kovalchuk[1]

1 *Zoology Department, Faculty of Biology, University of Uzhhorod, Ukraine*

Corresponding author: *Andriy Kovalchuk* (a_koval54@ukr.net)

Received 14 October 2022 | Accepted 18 May 2023 | Published 30 June 2023

Citation: Kovalchuk A (2023) The first sighting of *Isophya posthumoidalis* (Orthoptera, Tettigoniidae) in Ukraine and observations on its biology and acoustics. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa" 66(1): 113–133. https://doi.org/10.3897/travaux.66.e96322

Abstract

Isophya posthumoidalis is a rare species native to Central Europe that is only found in a very small area of Poland, Slovakia, and Romania. Since Bazyluk's description in 1971, it has been regarded as endemic to the Eastern Bieszczady mountains. In 1976, we discovered this species in Zakarpattia Province (Transcarpathia, Ukraine), but the records were not published, because there were no males available. Due to the analysis of a male's acoustical signals in 2022, further confirmation was obtained.

Keywords

Isophya, first record, rare species, bioacoustics.

Introduction

Multiple revisions (Heller et al. 2004; Bey-Bienko 1954, 1964; Harz 1969; Zhantiev et al. 2017; Lykovitch and Kovalchuk 2019) have reported ten species and subspecies of *Isophya* Krauss, 1902 in Ukraine, namely: *I. brunneri* Retowski, 1888., *I. camptoxypha* (Fieber, 1853), *I. doneciana* Bey-Bienko, 1954., *I. stepposa* Bey-Bienko, 1954., *I. stysi* Cejchan, 1957, *I. taurica* Brunner von Wattenwyl, 1878., *I. pienensis* Maran, 1954, *I. zubovskii* Bey-Bienko, 1954, *I. modesta rossica* Bey-Bienko, 1954, and *I. modesta modesta* Frivaldszky, 1868. In the Holyts'kyy Botanical Reserve, in Ternopil province (lat 49.40760, long 24.83289; June 27, 2002), located 160 km from the localities of

I. modesta in Pogórze Przemyskie, Poland (Czyżewski and Guzik 2022), we found *I. m. rossica* (1 ♂ ♂ and 1 ♀♀). Orci and Heller (2004) identified the material from the vicinity of the Kanev Reserve (Cherkasy province, 50 km from Kyiv) as *I. m. modesta* with some uncertainty. However, Bey-Bienko (1954, p. 223) doubted the not far affiliation of *Isophya modesta* specimens from the vicinity of Kyiv (Ramme 1951) and suggested that only *I. m. rossica* is possible in this area. Therefore, Bey-Bienko's (1954) view is that the occurrence of *I. m. modesta* in Ukraine is doubtful, but it is difficult to be certain. It's worth noting that the discovery of *I. m. modesta* in an unconventional location like the Kanev Reserve suggests the presence of a refuge for thermophilic species, which is supported by the citation of a bush-cricket *Poecilimon affinis* Friv. from the same area by Dobosh (1977).

However, due to their geographic location, natural conditions, and limited research, certain regions, such as Transcarpathia, may have a significantly higher number of bush-cricket species, including *Isophya*. The recent discovery of a new *Pholidoptera* species in Ukraine, as reported by Kovalchuk (2021), supports this hypothesis.

Isophya posthumoidalis was originally described from the Western Bieszczady Mountains (Beskydy Mountains in Ukrainian) by Bazyluk in 1971. It belongs to the *Isophya camptoxypha* complex, sharing common morphological features with six related species (Iorgu et al. 2017). This species is typically found at altitudes ranging from 700 to 1130 m and has been reported in Slovakia, where it is quite common in areas bordering Ukraine (Kočarek and Jeziorski 1999; Krištín 2022a). The distance between the nearest recorded location in Slovakia and the Transcarpathian habitat in Ukraine is less than 10 km. *I. posthumoidalis* is also known from Romania, where it was found near the Tisa River, approximately 2–3 km from the Tyachiv and Rakhiv districts of Transcarpathia (Szövényi and Orci 2008), where individuals of *I. posthumoidalis* have been recorded in hilly areas at altitudes ranging from 253 m to 369 m asl.

Material and methods

Study area.

The mapping of bush crickets in the "Vajdiv Luh" area was conducted during two periods, from 1976 to 1978 and from 1994 to 1996, at an altitude of 510–526 meters above sea level. This area serves as a natural border between the Uzhhorod and Perechyn districts of Transcarpathia and is situated on the western slopes of Mount Antalovetska Poliana (968 m), which is the final high mountain of the Gutin Volcanic Range stretching from Romania to the tectonic fault along the Uzh River near Slovakia (Fig. 1A). The mapping studies were irregular and not frequent due to the training operation of the tanks that started at the beginning of the 1970s, in this region. The grassland area in forest glades was significantly reduced (by more than 80%) as a result of tank tread damage to the grasslands, the abandonment of

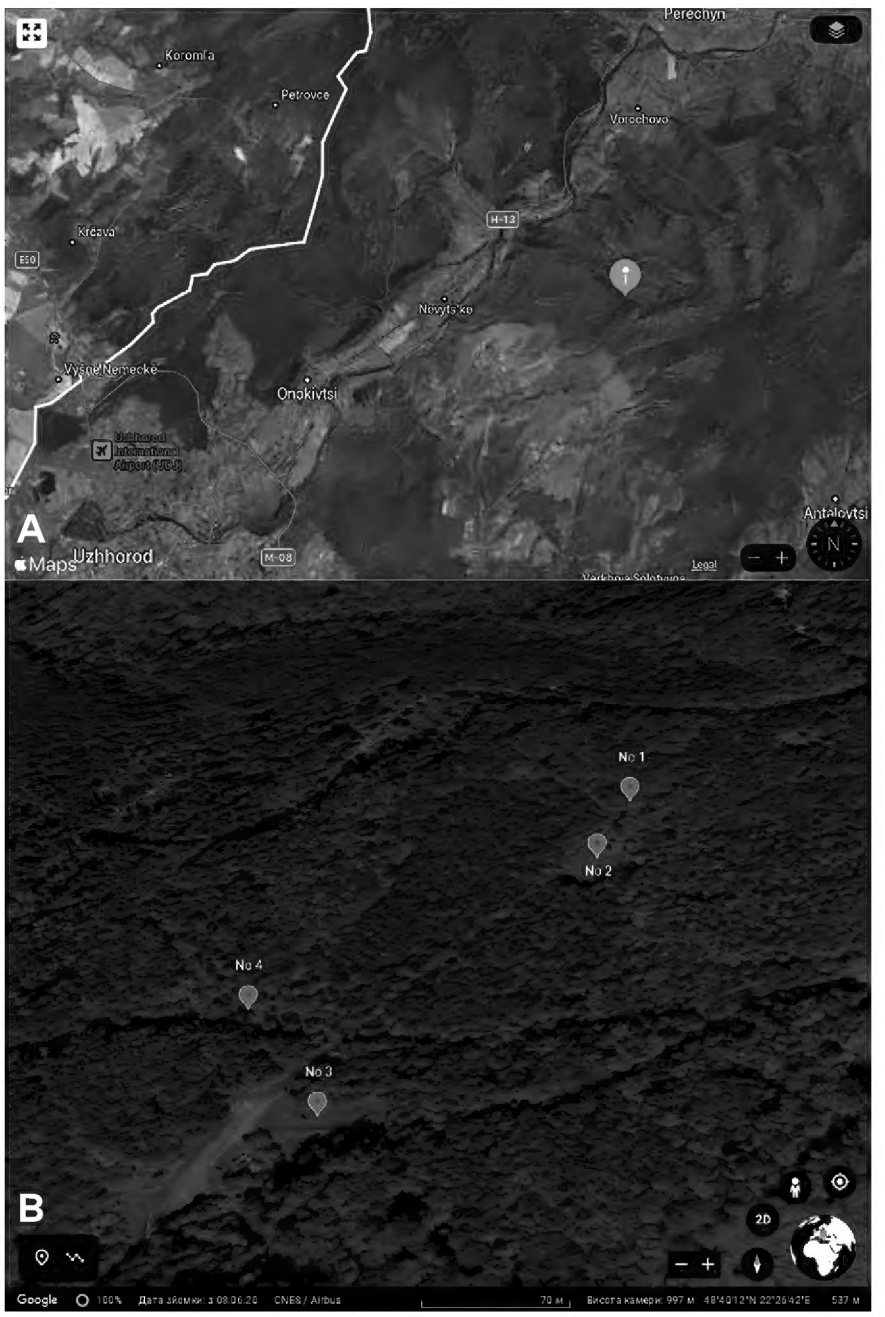

Figure 1. The location of "Vajdiv Luh" area: **A** a map of the districts of Uzhhorod and Perechyn (https://www.gps-coordinates.net/); **B** the location of clearings in the Vajdiv Luh tract (https://earth.google.com/). Red and blue marks indicate the location of the tract and clearings, respectively.

conventional mowing, and the subsequent planting of birch, willow, and beech. Currently, only four forest clearings (Fig. 1B) remain, which are spaced apart and can be considered favorable biotopes for bush-crickets Some of them (specifically 2–4) contained *Isophya* bush-crickets.

Glade #1 is a small area (approximately 15 × 15 m) that is overrun with bushes (48°40' 24"N; 22°26'55"E; altitude 526 m). In both glade #1 and glade #4 (48°40'16"N; 22°26'38"E; altitude 510 m), live large bush-crickets, such as *Pholidoptera transsylvanica* (Fischer, 1853) and *Tettigonia cantans* (Fuessli, 1775). *Isophya stysi*, a species known from Poland, Hungary, Romania and Slovakia (Bazyluk 1971; Orci et al. 2005; Iorgu and Iorgu 2008, 2011; Jarčuška et al. 2015; Krištín 2022b), is abundant in glade #2 (48°40'19 "N; 22°26'48 "E; altitude 503 m), glade #3 (48°40'13 "N; 22°26'40 "E; altitude 520 m), and glade #4. Since glades #2 and #3 (Fig. 2A) are hayfield-type meadows, regular mowing takes place in the second part (the last ten days) of July.

Due to the absence of conventional mowing, glade #4 (Fig. 2B) is overgrown with shrubs and young trees and may soon meet the same fate as glade #1.

Figure 2. The habitats of *Isophya posthumoidalis* in Ukraine (Vajdiv Luh) in July 2022: **A** glade #2; **B** glade #4.

Data collection

One male of *I. posthumoidalis*, two males and one female of *Isophya stysi* were used in the acoustic studies. All sampled specimens are in the author's private collection.

Equipment and experiments

This study was conducted with the BATSOUND TOUCH LITE software and a Pettersson M500 microphone. The microphone has a frequency response range up to 192 kHz. The HP Z-Book 15 mobile workstation running Windows 10 was used to store and analyse the acoustic signal. A TASCAM DR-40x portable recorder with a microphone frequency response of up to 48 kHz, was also used. The Dodotronic Ultramic UM250K (125 kHz) was used to capture sounds using SEAWAVE 2.0. We maintained these mics at least 20 cm to 1 m away from bug cages. The audio files were edited using ADOBE AUDITION 1.5 for Windows, AUDACITY 3.1.0, and AVISOFT-SASLAB LITE ver. 5.2.15. Images were taken using a Nikon D5200 camera in the field, and morphometric measurements of insects were made in the lab using a stereomicroscope MBS-1 equipped with a UCMOS 05100 KPA (x12) camera with 5.1 MP resolution and TOPVIEW 3.5.363 software. Screenshots were taken using the FASTSTONE CAPTURE software. Insects were kept in 5–10 l plastic cages with two opposite walls that had been cut out and coated with nylon mesh for the tests. Both audio-visual contact between specimens and solely auditory perception were applied to study the *Isophya* specimens. The statistical calculations were performed with STATISTICA 10 for Windows.

Experiments with acoustic species interaction were conducted in two ways: during audio-visual contact, where males of both species were in the same cage, and in the other case, the males of *I. posthumoidalis* and *I. stysi* were in separate cages, at a distance of 3 m from each other. Obviously, in such experiments only an audio contact can occur. A male *Pholidoptera transsylvanica* from Glade #4 was used in several tests, and its cage was put 1 m from an *Isophya posthumoidalis* cage and roughly 3 meters from the *Isophya stysi* cage.

We created about 60 song recordings of *Isophya posthumoidalis* and *I. stysi* males lasting 3–17 minutes, and about 500 short 15-second triggered recordings. Synchronous singing of *I. posthumoidalis* was detected for 15 series of syllables of *I. stysi* in 11 files.

Results and Discussion

We started investigations in Vajdiv Luh tract in 1974. At the time, glades #1–4 were in fact a single large meadow with no woody vegetation except for a few lone bushes. Some smaller individuals of *Isophya* were found during the monitoring of *Isophya stysi* local population, which after careful examination proved to be *Isophya posthumoidalis* (a total of 3 females were collected: 1♀♀ in July 25, 1976, 1♀♀ in June 26, 1978, 1♀♀

in July 25, 1976). Additionally, the author was unable to publish this study due to a lack of males *I. posthumoidalis* and comparable original material from Poland.

Later we investigated a male *Isophya posthumoidalis* and its song (from glade #2) in July 2022, in a location where the species has not previously been recorded over the course of four visits in the last three years (some after the grass has been mowed). So, *Isophya stysi* was found in glades #2, #3 and #4 (Fig. 3A), while *I. posthumoidalis* was found only in glades #2 and #4 are home to *alis* (Fig. 3B). But considering that glade #3 and glade #4 are less than 100 meters apart, it is quite likely that it also occurs there. The original Orthoptera fauna within each fragment was the same because all four glades were formerly one single large, continuous meadow. Later, the reduction of the meadow area, shrub overgrowth, the abandonment of traditional land use linked with haymaking, and landscaping of the region for a military tank training area could have caused *Isophya* and several other species to become extinct.

The first adults of *Isophya stysi* and *I. posthumoidalis* usually emerge in this region at the end of June. When we visited the glades on June 16, 2022, we observed a fairly large number of Isophya nymphs in glade #4 (Fig. 4), but adults were still absent. *I. posthumoidalis* probably survives longer in the conditions of glade #4 than *I. stysi* (until the middle of September). In fact, even at the beginning of October 2021, we observed a solitary female *I. posthumoidalis* in glade #4.

Notes about morphology. Isophya posthumoidalis (Fig. 5) stands apart from all other species in the region due to its small size, fine spotted body (one female appeared completely green), large and saddle-shaped male pronotum, short hind femur, and extremely short female ovipositor (Table 1). These findings are in agreement with data

Figure 3. Males of *Isophya stysi* and *Isophya posthumoidalis*: **A** *I. stysi* in glade #2; **B** *I. posthumoidalis* in the experimental settings; the red strip on the abdomen is number I (one) and was applied with a marker for easy recognition in the experiment.

Figure 4. Nymphs of *Isophya* in the wild (glade #4): **A** male; **B** female.

published by Bazyluk (1971) and Heller et al. (2004). Our second female's ovipositor was a little bit longer.

Isophya posthumoidalis males in Central Europe produce a distinctive and rhythmic song consisting of two types of syllables (Syls), which is also observed in some other species of *Isophya*. This type of song was first documented in *Isophya beybienkoi* Maran (Orci et al. 2001). Although descriptions of *Isophya dochia* (Iorgu 2012), *Isophya nagyi* (Szövényi et al. 2012), *Isophya sicula* (Orci et al. 2010), and *Isophya bucovinensis* (Iorgu et al. 2017) from Romania expanded the *Isophya* species with related morphology, but only *I. posthumoidalis* and *I. sicula* exhibit the same two-syllable singing pattern. Morphologically, *I. sicula* and *I. posthumoidalis* are identical and resemble *Isophya camptoxypha*, which is the species on the basis of which a morphological cluster was formed, including other recently discovered *Isophya* species with similar morphology but different songs.

Under test conditions, the song pattern of male *Isophya posthumoidalis* resembles the one described by Heller et al. (2004) and Orci et al. (2010). A discrete song unit is composed of syllables organized into series (Syl-S). Multiple series form groups (GS) separated by gaps. Therefore, the group of series includes several series, each usually composed of two semi-series (S-S). The first semi-series is a sequence of multiple "A"-type syllables, while the second consists of only one "B"-type syllable, such as ‹AAA…AB› (Table 2).

The mean duration of a GS, calculated from the logarithmic form of the log-normal data distribution, is 51 seconds, with maximum durations of up to 480 seconds being detected. This contrasts with the findings of Heller et al. (2004), who

documented a maximum GS duration of just 80 seconds. Typically, a GS consists of 12 Syl-S, but there have been instances where a GS has had as many as 124 Syl-S. The gap between two successive GS is twice the duration of a GS and follows a normally distributed data sample. It is clear that the length and quantity of Syl-S in GSs are influenced by various environmental factors such as weather and climate, as well as biocenotic audiovisual cues during intra- and interspecies interactions.

The mean duration of the "A"-type Syl is significantly longer than the "B"-type (T-test, $p<0.000001$) depending on more impulses in "A" Syl (Table 2). Rarely GS is initiated by 1–2 isolated Syls of "A"-type and is usually completed by 1–3 or even

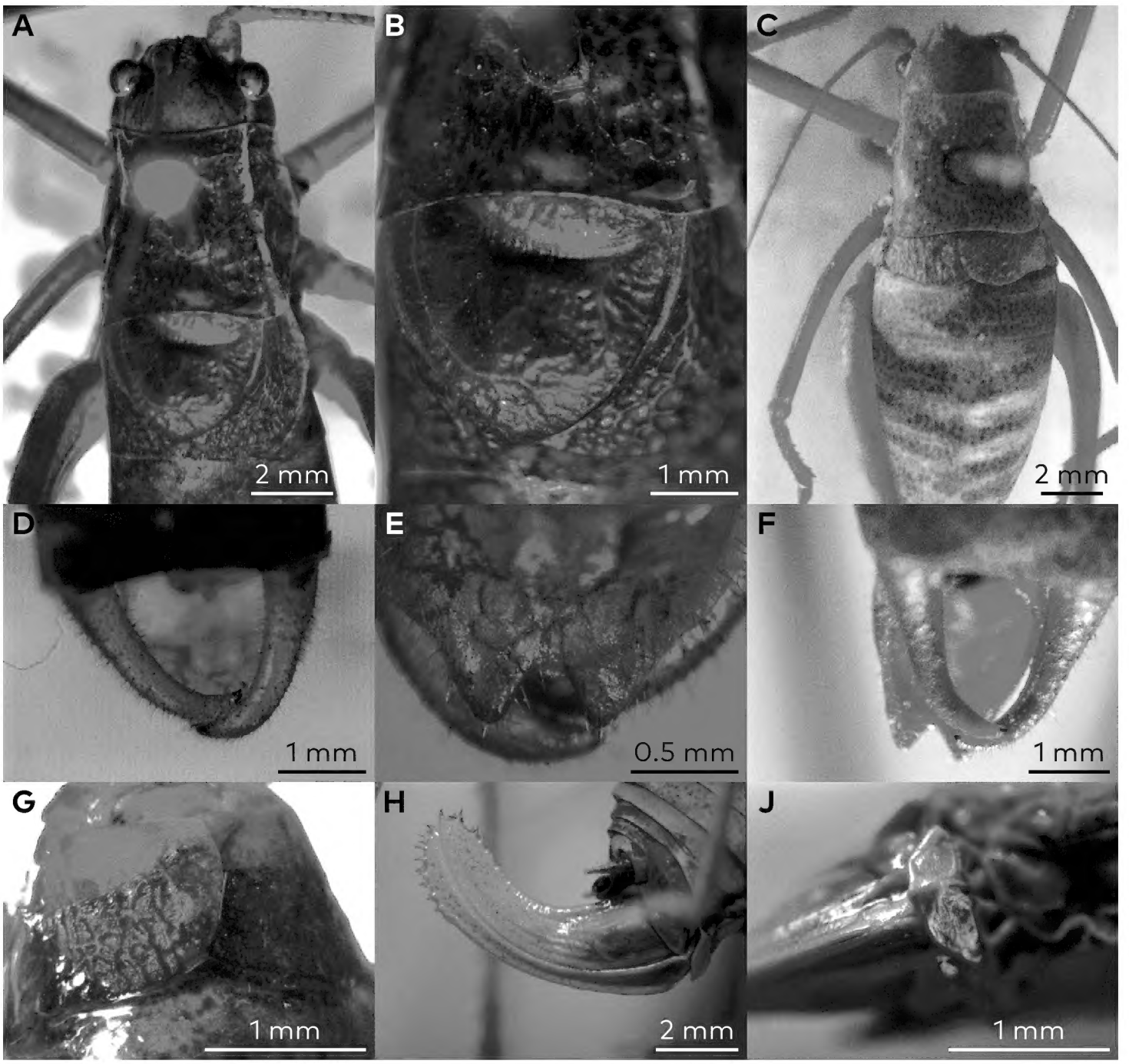

Figure 5. Body parameters of male and female *Isophya posthumoidalis*: **A** male head, pronotum, and tegmina (July 9, 2022); **B** full view of tegmina of a male; **C** body of the female 2; **D** cerci of a male from up; **E** subgenital incisura shape of a male from down (×48); **G** full view of tegmina of female 3; **H** ovipositor of the female 1; **J** female 1 subgenital incisura from down. *Isophya stysi*: **F** cerci and genital incisura of a male from up (July 1978, glade #4).

Table 1. Morphometric characters of *I. posthumoidalis* specimens.

Characters, mm	male	1st fem	2nd fem	3rd fem[3]
Width of head	3.5	4.1	4.1	-
Body size[1]	20.8	21.0	19.6	≈20.0
Length of pronotum	3.8	4.4	4.4	-
Width of pronotum	4.8	4.7	4.9	-
Length of left elytron	3.2	2.7	2.8	2.7
Width of left elytron	3,4	-	-	-
"Character 6"[2]	1.9	-	-	-
"Character 7"[2]	1,2	-	-	-
Length of hind femur	18.6	20.9	20.3	20.3
Length of left cercus	2.0	-	-	
Depth of subgenital incisura	0.3	-	-	
Width of subgenital incisura	0.7	-	-	
Length of ovipositor	-	6.9	8.0	7.0
Width of ovipositor	-	2.0	2.0	2.0

[1] – on dry material; [2] – left elytron characteristics (in Orci et al. 2005, fig. 2); [3] – damaged.

more "A" Syls, final or closing syllables, CS. "A"-type is a series of Syls, while "B" is a solitary Syl with a few after- clicks.

The entire song of *Isophya posthumoidalis* might well be codified as ‹AAA…A-B-AAA…A-B-AAA…A-B-AAA…A-B-AAA…A-B-AAA… A-B-AAA…B-FI›. However, at about 15% of cases, it ends with ‹-B-AAA…A›.

A syllable period (SP) is determined by the duration of a syllable and the gap between syllables. The beginning of the "B" S-S period is defined as the start of the "B" syllable until the initial syllable of the next "A" S-S. The distances between consecutive S-S and successive series are not statistically different based on T-test analysis. The initial syllables in the first "A" S-S ("A"-1st S-S) are usually atypical and have a much longer SP, so they are computed separately and presented in Table 2. Since the GS towards the end are longer than those at the beginning, we adopted the following approach for statistical calculations: for the conventional series "A", we calculated the last three series (n=3) and the other three starting with the second to fourth series (n=3), resulting in a total of six Syl-S in the GS.

We analyzed the frequency spectra of syllables (FS) using data from 50 Syl-S in seven GS recorded by an M-500 Mic and the same number by TASCAM RX-40 Mics. The recorded average maximum frequency data range between 23 and 24 kHz, and there is no significant difference between them (Table 2). While generally replicating the findings of Heller et al. (2004, fig. 90) for Polish *Isophya posthumoidalis* specimens, the FS displays one sizable, isolated maximum, with the second "spike" likely due to technical background noise. This second "spike" appears in both spectra of syllables

Table 2. Descriptive statistics for the studied characters of the male calling song of *Isophya posthumoidalis* (see Fig. 6 and the text for abbreviations; durations are in s).

Analysed characters	*n*	Mean	Min.	Max.	S.D.
Group of series (GS)					
Duration of GS	18	51(3.92*)	7	480	1.14*
A gap between two GS	15	121	38	300	70
Number of closing syllables (CS) in GS	20	2(0.87*)	1	17	0.96*
A gap from "B" syllable to 1st CS in final Syl-S	17	0.325	0.291	0.358	0.021
Syllable-series (Syl-S)					
Number of Syl-S in GS	18	12(2.46*)	1	124	1.32*
Max of FS in Syl-S, kHz; M500	50	23.8	23.2	25.2	0.3
Max of FS in Syl-S, kHz; DR-40x	50	23.4	21.3	24.5	0.9
Duration 1st Syl-S period	29	7.4	4.7	10.3	1.4
Interval between "A" and "B" S-S	28	0.302	0.234	0.346	0.022
"A"-1+n (typical) semi-series (S-S)					
Number "A" syllables in S-S	59	19	11	31	5
Duration "A" syllable	50	0.034	0.029	0.039	0.002
"A" syllable period	51	0.159	0.093	0.257	0.053
"A"-1st semi-series					
Number of "A" syllables "A"-1st S-S	29	43	27	58	9
"A"-1st S-S duration	29	6.9	4.1	9.7	1.4
NS in slow phase "A"-1st S-S	29	11	6	16	2
NS in fast phase "A"-1st S-S	29	32	15	48	8
Duration slow phase "A"-1st S-S	29	2.8	1.3	4.4	0.7
Duration fast phase "A"-1st S-S	29	4.1	1.8	7.0	1.2
"B"-1+n (typical) semi-series					
Duration "B" syllable	55	0.029	0.020	0.033	0.003
Period "B" S-S	55	0.350	0.320	0.381	0.012
After-clicks number in "B" syllable	55	1.673	0	2	0.579
"B"-1st semi-series					
Duration "B"-1st syllable	28	0.029	0.020	0.040	0.005
Period "B"-1st S-S	27	0.330	0.282	0.360	0.019
After-clicks number in "B"-1st syllable	26	1.808	0	3	0.567
Syllables					
Duration "A"-type	60	0.023	0.002	0.019	0.026
"A"-type NI	60	3.7	0.66	3	5
Duration "B"-type	21	0.017	0.001	0.016	0.19
"B"-type NI	21	2.8	0.70	2	4

* – log-normal meaning of data: $\log x_i$.

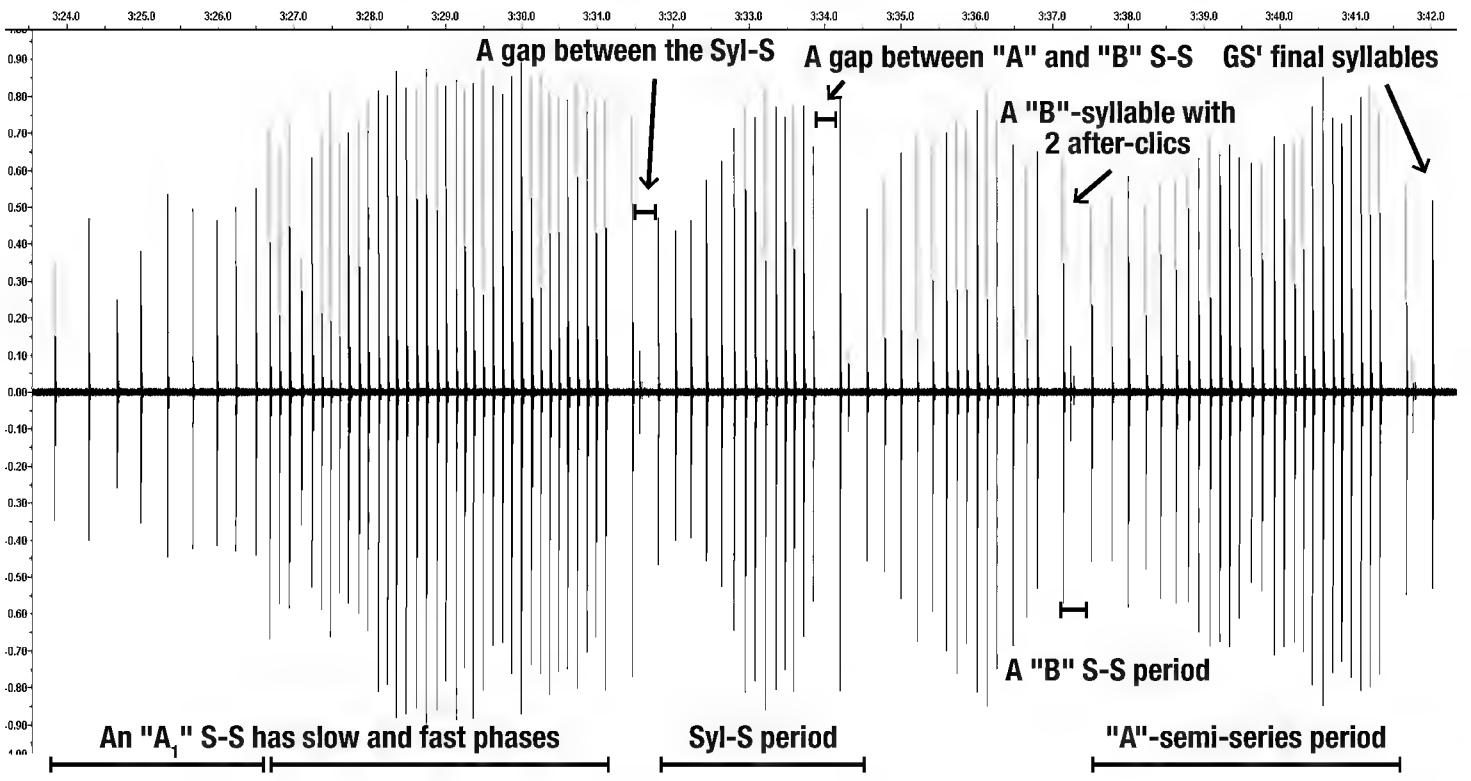

Figure 6. *Isophya posthumoidalis* male song characteristics: An oscillogram of a representative group of series (GS) containing four syllable series (SylS) with the investigated characteristics, created using Audacity.

that are very closely spaced in the common sonogram of both species' songs (the "A"-type Syl for *I. posthumoidalis*) during an audiovisual experiment (Fig. 7A, B).

Our analysis of the male calling song of *I. posthumoidalis* revealed several noteworthy results (Table 2). We observed a longer gap between the S-S "A" and "B" than the "A"-type syllable period duration. According to a T-test, the regular "B" S-S period is significantly longer than the "B"-1st S-S period. The duration of the "B"-1st impulse generated in the first series is equal to the regular "B"-n_i (where n>1) impulse produced in the following series (T-test). There is no significant difference in the number of after-clicks in the initial "B"-1st S-S and following "B" S-S in an SG (T-test). The duration of A-type Syls is also substantially longer than B-type Syls because they contain a significantly higher number of impulses (NI) than B-type Syls do.

Researchers have been interested in the acoustic interactions between different species of bush crickets for a long time (Regen 1914, 1926; Jones et al. 1966). Living in the same habitat means that specimens have audio or occasionally the closest audiovisual contacts. Does *Isophya stysi*, a species with a higher population density and more intense male song, have the ability to pause or even stop *I, posthumoidalis* male song completely? If yes, we can discuss interspecies competition.

Some very controversial results were obtained in the course of experiments with the synchronous singing of males of both species. It is a confirmed fact that singing in a duet by males of the same species is a common phenomenon. We have sufficient data on such singing for all species of *Isophya* found so far in Transcarpathia. The results of some experiments with *Isophya camptoxypha* males are now in press (Kovalchuk 2023). When singing in a duet, males of the same species coordinate their singing,

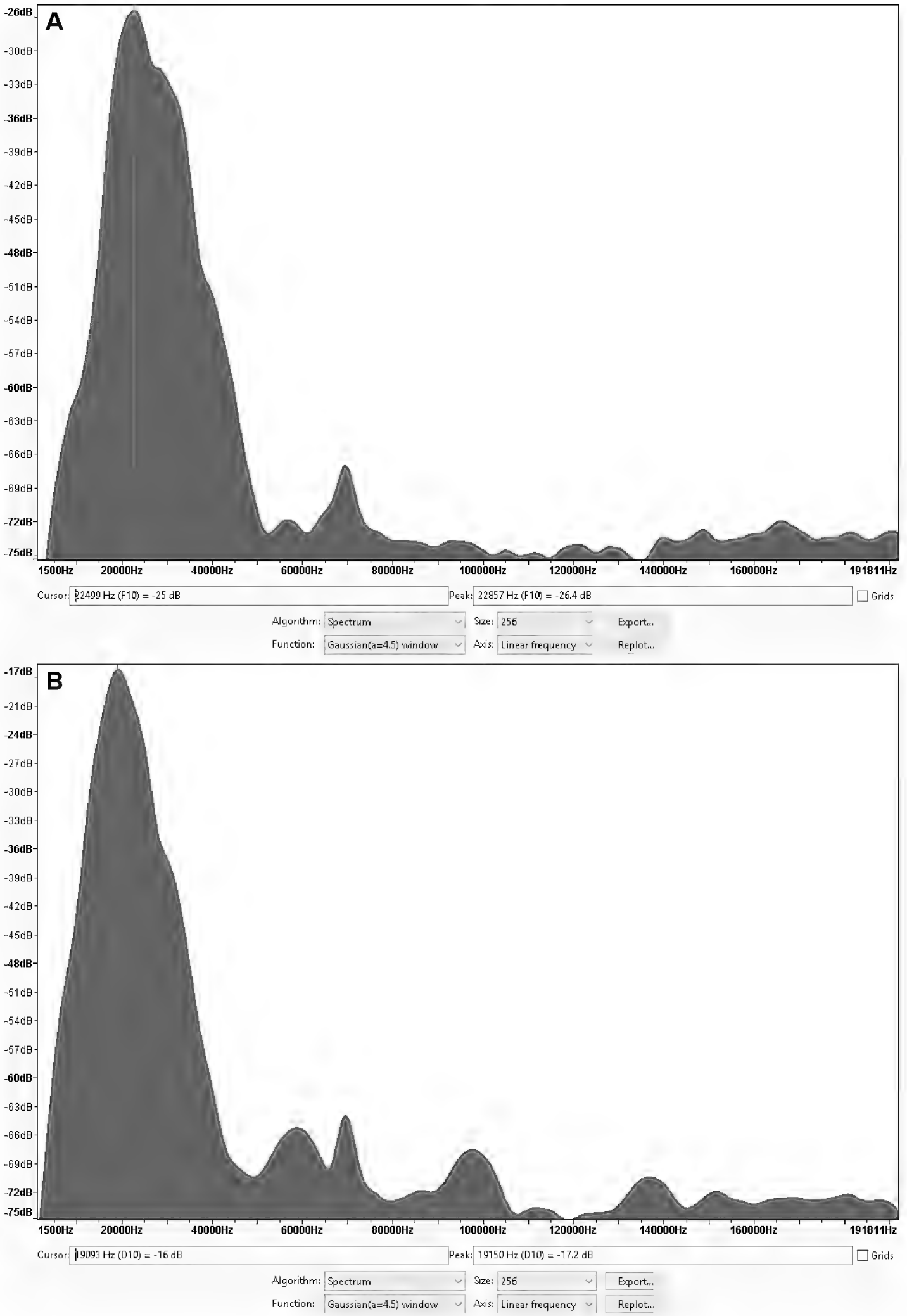

Figure 7. Comparison of male syllable spectra between *Isophya posthumoidalis* and *I. stysi.* **A** shows the spectra of male syllables of *I. posthumoidalis*, while **B** shows the spectra of male syllables of the first male of *I. stysi*.

trying not to overlap each other's syllables (Fig. 8A), which reflects the ability of one male to play the dominant role while the other remains subordinated. This is most likely a defence mechanism against confrontation in intraspecies competition. The

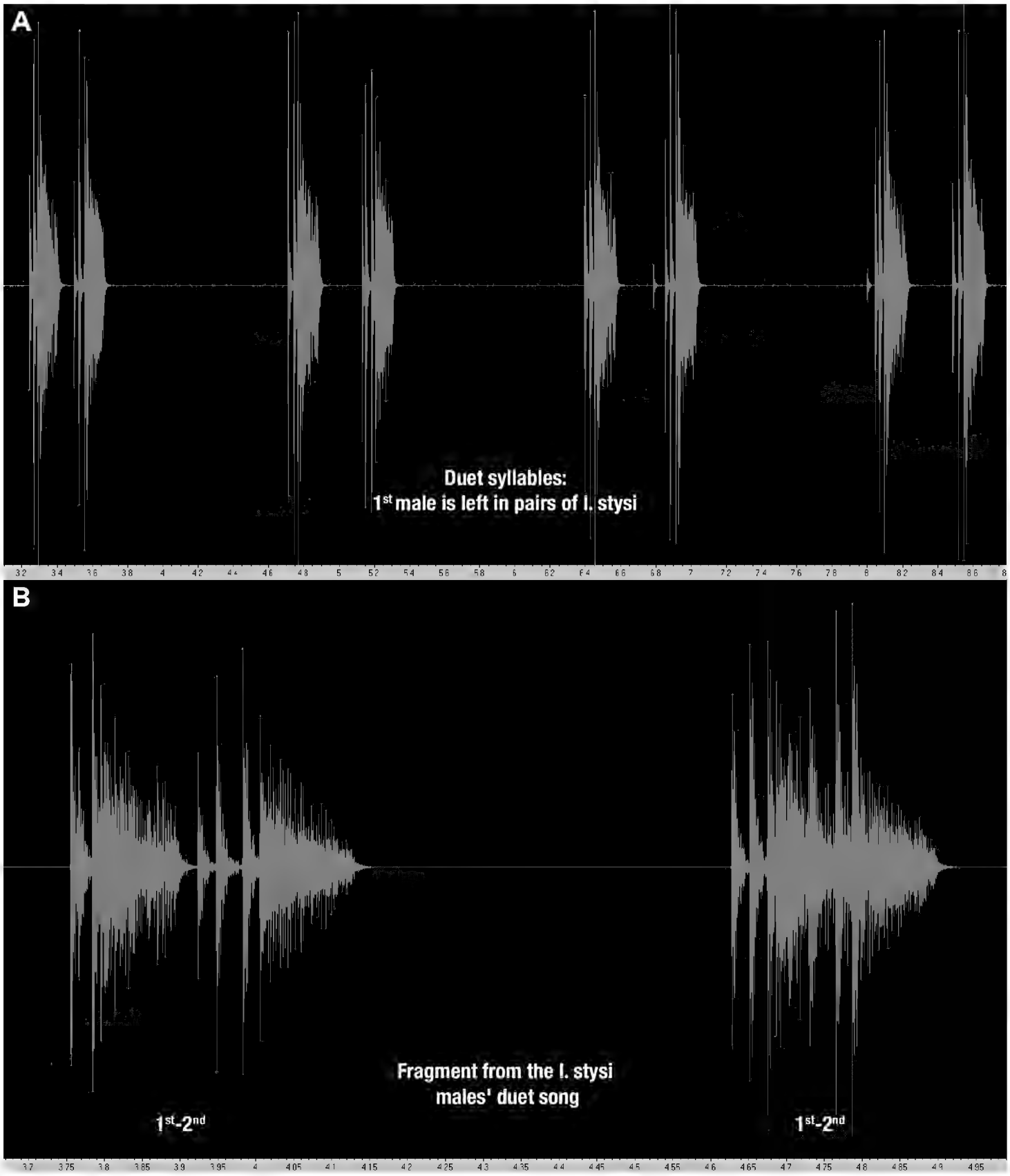

Figure 8. *Isophya stysi* males singing a duet with doubled syllables during intraspecific interaction while in audio-visual contact. In each pair, the lead male (as 1st) is on the left. The oscillogram was transformed to 44.1 kHz frequency using Adobe Audition 1.5. **A** represents the standard or "proper" singing, while **B** represents a duet with unusual syllable closeness (left) or overlap. The gap between syllables in option **B** is slightly reduced for clarity.

distance between syllable pairs can occasionally become smaller or even overlap for a variety of causes (Fig. 8B). In our tests, the situation returned to normal in 2–3 seconds.

In the Transcarpathian region, there are historical records of male *I. stysi* singing from the outskirts of Uzhhorod district (Zhantiev et al. 2017). In our experiments the singing of *I. stysi* males in duet has been observed to form consecutive duet syllable, where the syllables of each male typically do not overlap, as mentioned before. However, when males of different species come into contact, there are only three possible outcomes: inertia, avoidance, or blocking the opponent's activity, as we have analyzed for invertebrates in previous studies (Kovalchuk 2017). In the forest glade, where options for avoiding a rival are limited, distance from a potential competitor is almost the only type of protective activity in coexistence conditions.

When *Isophya posthumoidalis* sings at a distance of 3m from *Isophya stysi*, it is most likely a "non-contact" situation, whereas audiovisual singing clearly constitutes "contact". However, it is important to determine the nature of this contact. One effect of *I. stysi* on *I. posthumoidalis* during contact singing can be observed in the GS duration (Fig. 9A). The structure of the syllables of both species in contact singing does not appear to change (Figs. 9B–9D), but more research on statistically reliable data is needed to draw definitive conclusions. Therefore, it remains to be seen through further experiments *in vitro* and *in natura* whether *Isophya stysi* has the ability to pause or even stop *Isophya posthumoidalis* male song completely, which could indicate possible interspecies competition.

During synchronized singing, the songs of both *Isophya posthumoidalis* and *Isophya stysi* can undergo changes, which are audible in the number of syllables and their appearance or absence. However, only audiovisual experiments provided evidence of any signs of influence. In a distance-based experiment set at 3 m, the song of *I. posthumoidalis* did not exhibit any noticeable alterations (Table 3, series 9–11). We may only discuss a single instance of a related phenomenon, as depicted in Figure 10A and Table 3, Series 10-2, although there may be other underlying reasons. Furthermore, in a related experiment with *Pholidoptera transsylvanica* at a distance of 1 m, the song of *I. posthumoidalis* did not change (Fig. 10B).

When in audiovisual contact, the signal strength of a single syllable of *I. stysi* is higher than that of *I. posthumoidalis*, as indicated by Fig. 7 (around minus 15–17 dB compared to minus 25–27 dB). However, *I. posthumoidalis* has a higher syllable count per second. Therefore, it is reasonable to assume that there is a possibility that the two species influence each other. *I. stysi* males sang much less frequently when *I. posthumoidalis* was singing than when it wasn't. Additionally, the series of *I. stysi* songs recorded in audiovisual contact with *I. posthumoidalis* was significantly shorter. Without contact, a male *I. stysi* produced series with syllable counts ranging from 1 to 13 (6.0±2.7, n=28), whereas the male with contact singing produced series with syllable counts ranging from 1 to 5 (2.8±1.5, n=12, Figs 10A, B). Thus, the number of syllables in the series decreased by nearly half for the *I. stysi* male that sang in contact.

The question remains whether this effect is solely based on loud sound, which can interfere with the singing of the male *I. posthumoidalis*, or whether the male *I. posthumoidalis* can actually identify the singing of the male *I. stysi*. Further experiments are necessary to distinguish between these possibilities. If the latter is true, the response will have a much lower threshold of sensitivity than the former.

During audiovisual contact at close distance, certain deviations in the song structure of *I. posthumoidalis* were observed (Table 3). For example, a break in the semi-series "A"-type was noted. Even after the break, the male *I. posthumoidalis* still performed semi-series "B"-type quite often, in 10 out of 18 cases (Fig. 9A). Moreover, "B"-type syllables were accompanied by a smaller number of after-clicks. However, the interruption of the *I. posthumoidalis* series during *I. stysi* singing did not lead to a complete cessation of singing. Most often, the singing continued in the usual manner, with some cases after the opponent's syllable even starting a series similar to the first series in a given session, even with a noticeable slow period of singing at the beginning.

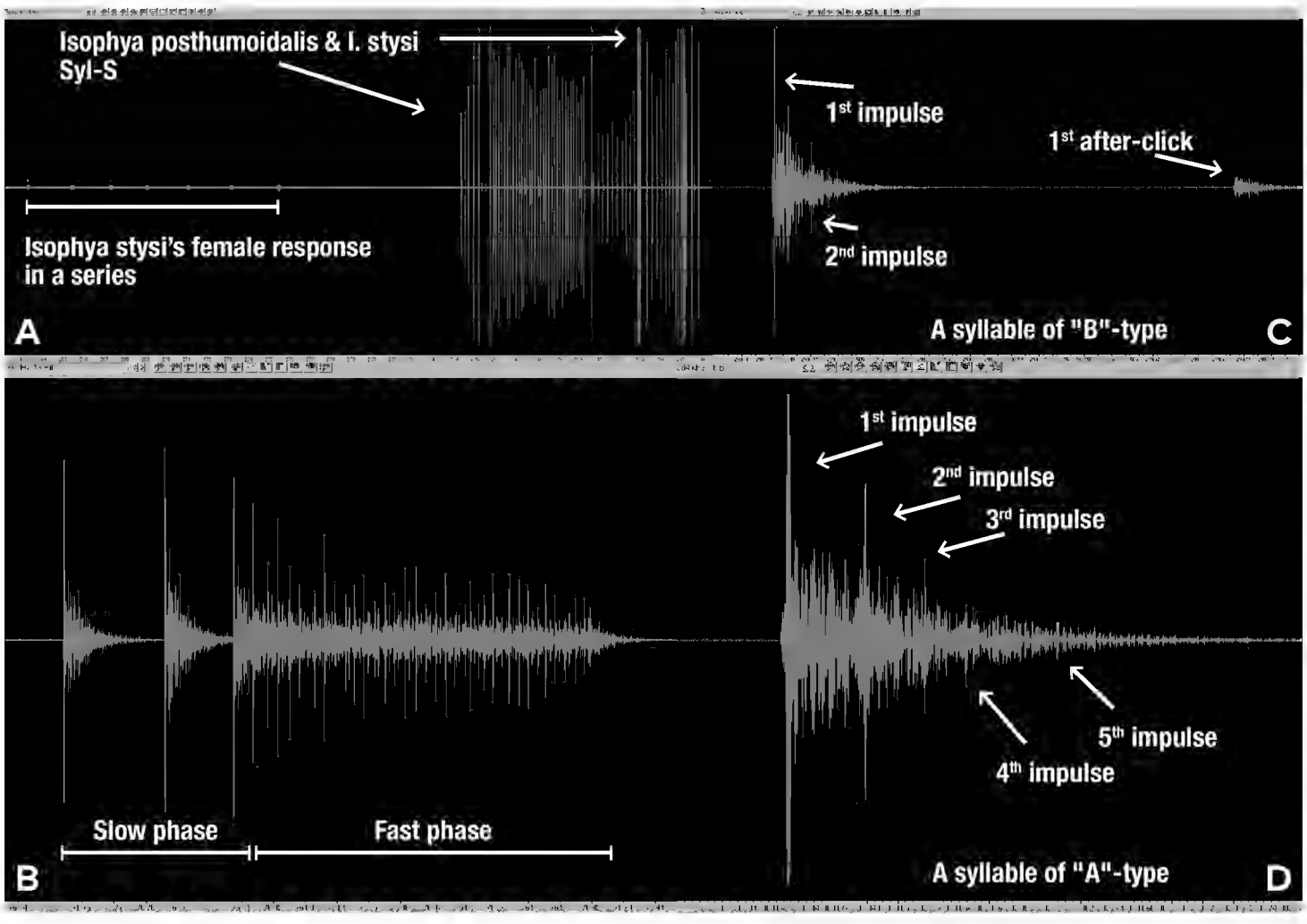

Figure 9. Oscillogram of a male *Isophya posthumoidalis* and specimens of *Isophya stysi* (all in AVISOFT): **A** experiment with close audiovisual contact and without interruption of singing but with a much shorter synchronous syllable's series and absence of the final "B" type S-S of *I. posthumoidalis* (M500); two syllables of *I. stysi* are red; **B** a syllable of the 1st male *I. stysi* (TASCAM DR-100, 48 kHz); **C** a "B"-type; **D** an "A"-type syllables in *I. posthumoidalis* male's song (M500).

Table 3. Deviations in the songs of *Isophya posthumoidalis* and *Isophya stysi* males during synchronous singing.

I. stysi's song																		
Type of a contact	Audiovisual																	
Series #	1-1	1-2			1-3			1-4		2-1		3-1		4-1		5-1		6-1	
Syls in series	1	2			5			1		2		1		4		4		4	
Syls' # in a series	1*	1	2	1	2-3	4	5	1	1	2	1*	1	2-4	1	2-4	1*	2-4		
I. posthumoidalis' song in time of the appearance of *I. stysi*'s syllable																			
Breaks series	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-		
"B"-type S-S	+	+	-	-	+	+	+	-	-	+	-	-	-	+	-	+	-		
"B" S-S a-clicks	+	+	-	-	+	+	+	-	-	+	-	-	-	+	-	+	-		
New series	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-	-		
New 1st series	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Final syllables	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-		
Stopped SG	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-		

I. stysi's song																			
Type of a contact	Audiovisual													Audio-3m					
Series #	7-1			8-1			8-2			9-1				10-1	10-2		11-1		
Syls in series	4			4			2			5				1	1		3		
Syls' # in a series	1	2	3-4	1	2	3-4	1*	2	1	2	3	4-5		1	1	1	2	3*	
I. posthumoidalis' song in time of the appearance of *I. stysi*'s syllable																			
Breaks series	+	+	-	+	+	-	+	-	-	-	-	-		-	-	-	-	-	
"B"-type S-S	+	-	-	+	-	-	-	-	-	+	-	+		+	+	+	+	+	
"B" S-S a-clicks	+	-	-	+	-	-	-	-	-	?	-	?		-	+	+	+	+	
New series	-	-	-	+	-	-	-	-	-	+	+	+		+	-	+	+	+	
New 1st series	+	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	
Final syllables	-	+	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	
Stopped SG		+	-	-	+	-	+	-	-	-	-	-		-	+	-	-	-	

* – pre-click in *I. stysi*'s syllable.

During synchronous singing with *Isophya stysi*, *I. posthumoidalis* singing ceased entirely in only 7 cases. In 4 cases, the singing even ended with the emergence of the final, or an CS, i.e., "classical" series of syllables (Table 3). If such a break in singing occurred, it typically happened when the first or, less frequently, the second syllable of the *I. stysi* song's series of syllables emerged.

With longer Syl-S of *Isophya stysi* and shorter syllable periods, *I. posthumoidalis* cessation occurred more frequently (Fig. 11A). It is unclear why *I. posthumoidalis* stopped singing in one case but not in another, despite both species singing simultaneously under the same conditions (Fig. 11B). However, in the latter case, the reaction of *I. posthumoidalis* still exists and is manifested in a short-term change in the amplitude of the oscillogram.

It remains unanswered to the author how *I. posthumoidalis* was not found in the Vaidiv Lug area by other researchers during many years of research in Transcarpathia, as the first record of *I. stysi* occurred there and was identified by Grigory Bey-Bienko

himself in St. Petersburg (personal communication of Ivan Lykovych). At the time, *I. posthumoidalis* was far less common than *I. stysi*, and males were much scarcer than females. Notably, observing a mix of populations of two distinct *Isophya* species in the wild in Transcarpathia is uncommon; such a phenomenon has only been observed for *Isophya pienensis* Maran and *Isophya* camptoxypha (Fieber) in the higher forest zone in the Beskydians and the Polonynskiy Massif (Lykovitch and Kovalchuk 2019, and several personal records in 2022). These species typically do not share habitats even in the event of contact. Furthermore, there is no record of a second species of

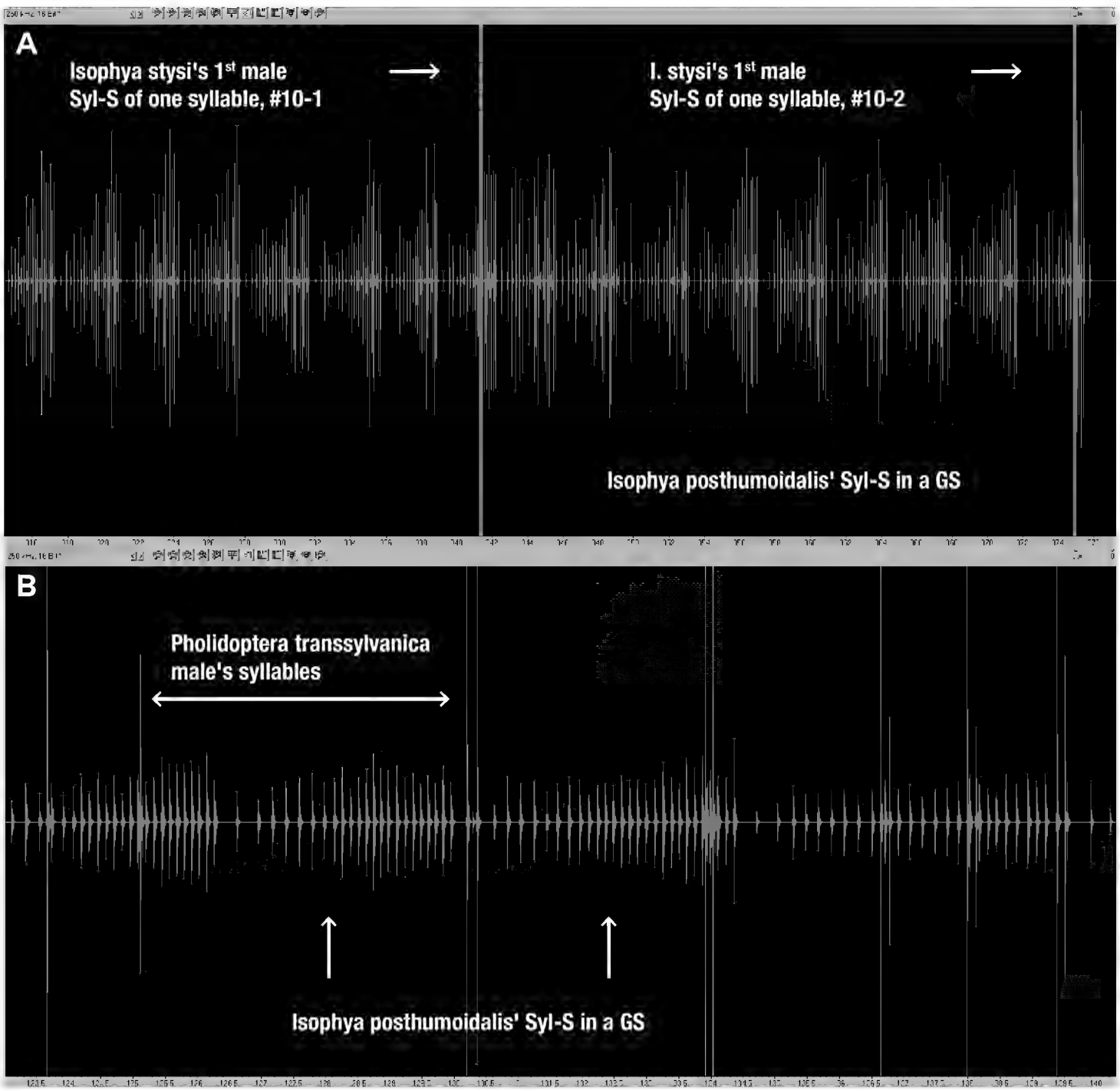

Figure 10. Oscillograms of male *Isophya posthumoidalis*, *Pholidoptera transsylvanica,* and *Isophya stysi* during the synchronous singing: **A** experiment with exclusive sound contact between *I. posthumoidalis* and *I. stysi* (distance about 3 m and height difference of 1 m; UM250K, AVISOFT); **B** experiment with possibly only exclusive sound contact between *I. posthumoidalis* and *P. transsylvanica* (distance about 1 m; UM250K, AVISOFT).

the genus in any of *I. stysi*'s Transcarpathian habitats (Szanyi 2014; Szanyi et al. 2015; Lykovitch and Kovalchuk 2019).

In contrast, in suitable conditions, even three species may appear together (Vadkerti and Szövényi 2005). The discovery of *I. posthumoidalis* is the last important link in understanding the geographical distribution of this rare Central European species. It is now clear that *I. posthumoidalis* is partially confined to the Gutin-Vyhorlat mountain range and further penetrates the Polish Bieszczady through Slovakia. This species is not particularly dependent on the altitude of potential habitats, as there is

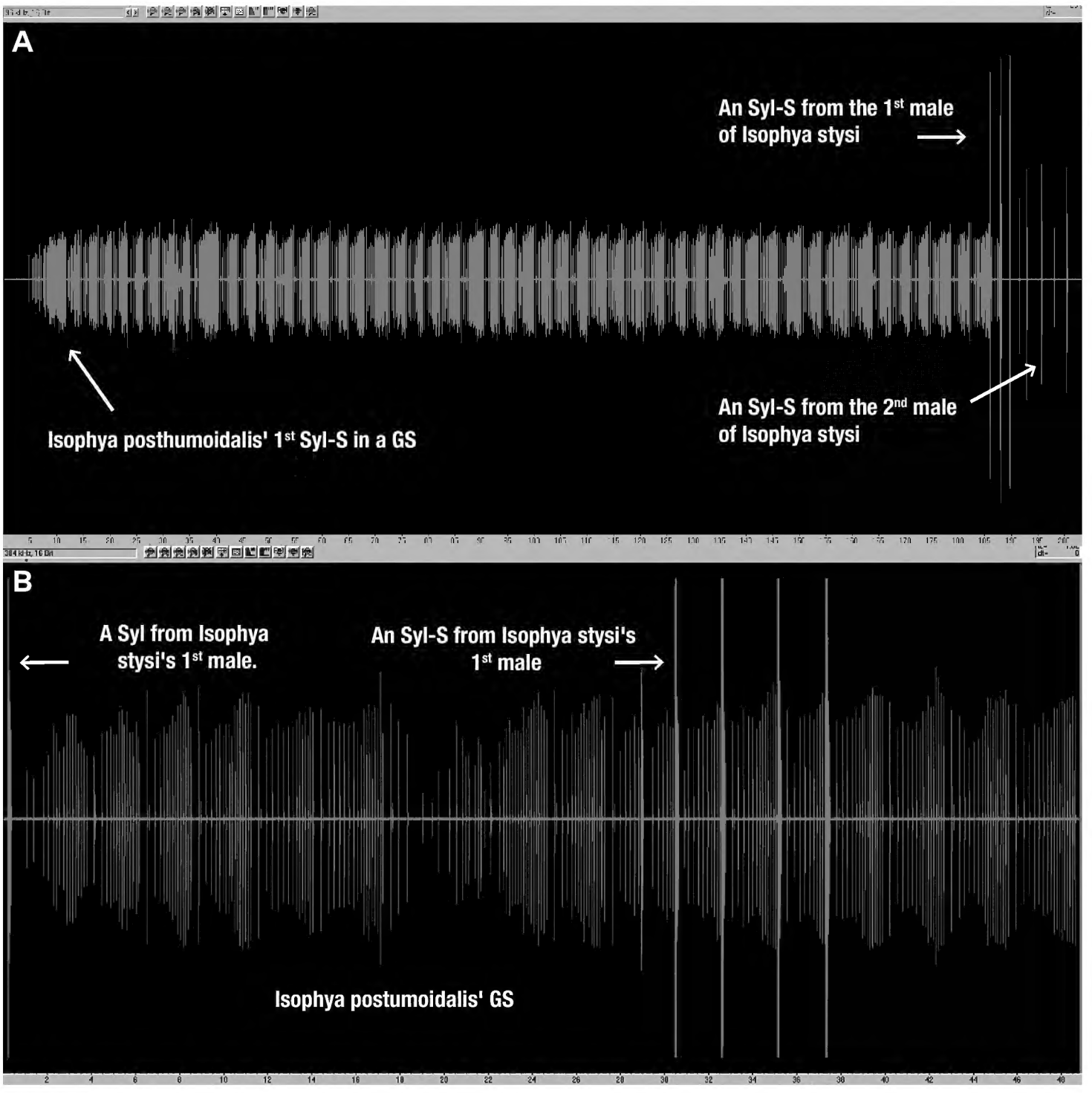

Figure 11. Oscillograms of male *Isophya posthumoidalis* and males of *Isophya stysi* during interspecies interactions: **A** experiment with close audio-visual contact and blocking of singing (TASCAM, AVISOFT); **B** experiment with close audio-visual contact and visible influence on amplitude, but without interruption of singing (M500, AVISOFT).

a significant difference in altitude in the habitats where it has been recorded, similar to other representatives of the 'camptoxypha' group that belong to montane species.

Conclusions

The discovery of *Isophya posthumoidalis* in Ukraine is an exciting development in our understanding of the geographical distribution of this rare Central European species. It is clear that the species is confined to the Gutin-Vyhorlat mountain range and penetrates the Polish Bieszczady through Slovakia. However, there is still much we do not know about its distribution and behavior.

The fact that the songs of *I. posthumoidalis* do not differ significantly from available data for individuals from Poland suggests that the species maintains a relatively stable song structure across its range. However, the observed temporary changes in the male singing of *I. posthumoidalis* when singing together with males of *Isophya stysi* indicate that there may be some degree of interaction between the two species, particularly in close audiovisual contact.

The co-occurrence of *I. posthumoidalis* and *I. stysi* is an interesting phenomenon, particularly given that it is uncommon to observe a mix of populations of two distinct *Isophya* species in the wild in Transcarpathia. This suggests that there may be some ecological or behavioral factors at play that allow these two species to coexist in certain habitats. Further research is needed to investigate this phenomenon and to gain a better understanding of the factors that shape the geographical distribution and behavior of these two species.

Acknowledgements

I am very grateful to the reviewers of my modest work, as well as to the responsible editor, Dr. Ionuț Ștefan Iorgu, who was very attentive to the situation and provided maximum assistance to the study, which was completed and prepared for publication in this difficult years for Ukraine. Many thanks for checking English of my friend from GB, Maria Naslidnyk.

References

Bazyluk W (1971) Prostoskrzydłe (Orthoptera) Bieszczadów Zachodnich wraz z opisem *Isophya posthumoidalis* n. sp. Fragmenta Faunistica 17(6): 127–159. [in Polish]

Bey-Bienko GY (1954) Orthoptera Vol. II, Sect. 2. Bushcrickets. Subfam. Leaf Bush-crickets (Phaneropterinae). In: Fauna U.S.S.R. (NS) 59. Institute of Zoology Academia of Science URSS, 385 pp. [in Russian]

Bey-Bienko GY (1964) Order Orthoptera (Saltatoria). In: Guide to insects of the European part of the U.S.S.R., volume 1. "Nauka", Moscow-Leningrad, 205–233 [in Russian]

Czyżewski S, Guzik P (2023) *Isophya modesta* Frivaldszky, 1868 (Orthoptera: Tettigoniidae): a new bush-cricket species in Poland. Fragmenta Faunistica 65 (1): 85–94.

Dobosh IG (1977) *Poecilimon affinis* Friv. – a new species of grasshopper for the USSR fauna. Vestnik zoologii 1: 86–87 [in Russian]

Harz K (1969) Die Orthopteren Europas. Vol. I. Dr. W. Junk N.V., The Hague, 749 pp. [in German]

Heller K-G, Orci KM, Grein G, Ingrisch S (2004) The *Isophya* species of Central and Western Europe (Orthoptera: Tettigonioidea: Phaneropteridae). Tijdschrift voor Entomologie 147: 237–258.

Jarčuška B, Kaňuch P, Krištín A (2015) Orthoptera and Mantodea assemblages of East Carpathian Mts (Central Europe). Folia faunistica Slovaca 20(2): 167–182.

Jones MDR (1966) The acoustic behaviour of the bush cricket *Pholidoptera griseoaptera*. Journal of Experimental Biology 45: 15–30.

Iorgu EI, Iorgu IȘ (2008) Bush-crickets, Crickets and Grasshoppers from Moldavia (Romania). PIM, Iași, 294 pp.

Iorgu EI, Iorgu IȘ (2011) Distribution of *Isophya stysi* in Romania (Orthoptera: Phaneropteridae). Conference: Annual Zoological Congress of "Grigore Antipa" Museum, Bucharest, Romania. doi 10.13140/RG.2.2.27049.39525.

Iorgu IȘ (2012) Acoustic analysis reveals a new cryptic bush–cricket in the Carpathian Mountains (Orthoptera, Phaneropteridae). ZooKeys 254: 1–22. doi 10.3897/ zookeys.254.3892

Iorgu IȘ, Iorgu EI, Szövényi G, Orci KM (2017) A new, morphologically cryptic bush-cricket discovered on the basis of its song in the Carpathian Mountains (Insecta, Orthoptera, Tettigoniidae). ZooKeys 680: 57–72.

Kocarek P, Jeziorski P (1999) First record of *Isophya posthumoidalis* (Orthoptera, Tettigoniidae, Phaneropterinae) from Slovakia. Biologia 54(2): 158.

Kovalchuk A (2017) Biocenotic Relationships and the Geographic Distribution and Conservation of Protozoa and Invertebrates. In: Biological Conservation in the 21st Century: A Conservation Biology of Large Wildlife Chapter: 8. Publisher: Nova Science Publishers Inc., 191–227.

Kovalchuk A (2021) The first find for Ukraine the littoral bush-cricket *Pholidoptera littoralis* (Fieber, 1853) from Transcarpathia. In: Current problems in the study of entomofauna of the western region of Ukraine. Publisher: L'viv, State Natural Museum of National Academic Sciences, Ukraine, 17–18.

Kovalchuk A, Pushkar T (2023) Exploring the singing behavior of male *Isophya camptoxypha* bush crickets (Orthoptera: Tettigoniidae) in the Uzh River valley: preliminary findings. Visnyk Lvivskoho Universytetu, Seriia biolohichna 89 [in Press]

Krištín A (Ed.) (2022a): *Isophya posthumoidalis*. Orthoptera of Slovakia. http://orthoptera.sk/ [accession date, 27.08.2022]

Krištín A (Ed.) (2022b): *Isophya stysi*. Orthoptera of Slovakia. http://orthoptera.sk/

Lykovitch I, Kovalchuk A (2019) Orthopteroids of Transcarpathia (Ukraine) – Part 1: historical excurse and present state with the main attention on Tettigonoidea. "Lira", Uzhgorod, 102 pp.

Orci KM, Szövényi G, Nagy B (2001) Description of the song of *Isophya beybienkoi* (Orthoptera, Tettigonioidea). Biologia 56(5): 489–495.

Orci KM, Heller K-G (2004) A description of the song of *Isophya modesta modesta* (Frivaldszky 1867) with notes on its relationship to *I. modesta rossica* Bey-Bienko, 1954. Journal of Orthoptera Research 13(2): 211–219.

Orci KM, Nagy B., Szövényi G, Racz I A, Varga Z (2005) A comparative study on the song and morphology of *Isophya stysi* Cejchan, 1958 and *Isophya modestior* Brunner von Wattenwyl, 1882 (Orthoptera, Tettigoniidae). Zoologischer Anzeiger 244: 31–42.

Orci K, Szövényi G, Nagy B (2010) *Isophya sicula* sp. n. (Orthoptera: Tettigonioidea), a new, morphologically cryptic bush-cricket species from the Eastern Carpathians (Romania) recognized from its peculiar male calling song. Zootaxa 2627: 57–68.

Ramme W (1951) Zur Systematik, Faunistik und Biologie der Orthopteren von Südost-Europa und Vorderasien. Mitteilungen aus dem Zoologischen Museum, Berlin 27: 8–319.

Regen J (1914) Untersuchungen über die Stridulation und das Gehör von *Thamnotrizon apterus* Fab. ♂. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe, Wien 123(1): 853–892. [in German]

Regen J (1926) Über die Beeinflussung der Stridulation von *Thamnotrizon apterus* Fab. ♂ durch künstlich erzeugte Töne und verschiedenartige Geräusche. Sitzungsber. der Akad. der Wissensch. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe, Wien 135: 329–368. [in German]

Szanyi S (2014) Preliminary data on the orthoptera fauna of the Beregszász hills (Transcarpathia, Ukraine). Tájökológiai Lapok 12(1): 107–115. [in Hungarian]

Szanyi S, Katona K, Rácz I, Varga Z, Nagy A (2015) Orthoptera fauna of the Ukrainian part of the Bereg Plain (Transcarpathia, Western Ukraine). Articulata 30: 91–104.

Szövényi G, Orci K (2008) Contributions to the Orthoptera fauna of Maramureş County with the first record of *Isophya posthumoidalis* in Romania 2008. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii (Life Sciences Series), 18 (suppl.): 235–241.

Szövényi G, Puskás G, Orci KM (2012) *Isophya nagyi*, a new phaneropterid bush-cricket (Orthoptera: Tettigonioidea) from the Eastern Carpathians (Caliman Mountains, North Romania). Zootaxa 3521: 67–79.

Vadkerti E, Szövényi G (2005) Habitat preference of four protected bush-cricket species (Orthoptera, Phaneropteridae, *Isophya*) in South Hungary. Biologia Bratislava 60/5: 545–549.

Zhantiev R, Korsunovskaya O, Benediktov A (2017) Acoustic signals of the bush-crickets *Isophya* (Orthoptera: Phaneropteridae) from Eastern Europe, Caucasus and adjacent territories. European Journal of Entomology 114: 301–311.